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SOME EFFECTS OF CADMINUM ON CONIFEROUS SOIL/LITTER MICROCOSMS

Harold Bond, et al

National Ecological Research Laboratory
Corvallis, Oregon

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SOME EFFECTS OF CADMIUM ON CONIFEROUS FOREST SOIL/LITTER MICROCOSMS

by

Harold Bond
Bruce Lighthart
Raymond Shimabuku
Loren Russell

National Ecological Research Laboratory
National Environmental Research Center
Corvallis, Oregon

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This report was submitted in fulfillment of ROAP 21ALU, Task 3, by the National Ecological Research Center. Work was completed as of February, 1975.

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Some Effects of Cadmium on Coniferous Soil/Litter Microcosms

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SECTION I
CONCLUSIONS

Overall microcosm systems design was satisfactory for detecting treatment effect differences in carbon dioxide generation and oxygen consumption over time. Albeit, the oxygen generation system was effected by barometric pressure shifts, particularly as waves of winter fronts passed through the area. They were presumed to average out over time.

Several aspects of the thermal system proved unsatisfactory and are the reasons for deletion of thermal data from this report. First, the thermister occasionally "burned out"; whether this was due to their moisture permeability is problematical. In any event, two parallel ceramic thermisters are to be used in future systems. It is imperative that each thermister as well as each reactor module be carefully calibrated for heat production and loss rate constant respectively! Finally, it is necessary to produce or account for any heat losses due to water vapor escapement from the microcosm/Dewar system, and temperature differential of the replacement carbon dioxide adsorbing solution in order to measure the biological heat production.

A future communication is in preparation and will detail the design improvements used to solve the problems just mentioned.

Similarly prepared and incubated soil/litter microcosms had an initial adjustment period after preparation that lasted approximately a week to ten days. The adjustment period is seen at both "indicator" and organismal levels as a relatively rapid respiration rate, i.e., oxygen utilization, carbon dioxide generation rate and microbial and nematode numerical increases. After the adjustment period, respiration decreases without a concomitant decrease in organisms indicating a slowdown of organismal activity presumably to a level more conducive to long-term survival at

low nutrient levels. For the microbes, this would be expressed by Winogradski as zymogenous growth of the fraction of the autochothonous population feeding on the altered nutrient supply following microcosm preparation from undisturbed soil. It is speculated that the zymogenous population in these experiments would be those autochothonous organisms that have relatively high growth rates (μ_m) and substrate saturation (K_s) constants (Jannasch, 1968, 1974) allowing their numerical increase in relatively high nutrient levels over those adjusted for lower nutrient levels. Upon depletion of the readily utilized substrate, the zymogenous population would slowly starve as reserves were exhausted, thus the populations would revert to the original autochothonous phenotypes equipped to operate at low nutrient concentrations, i.e., low K_s and μ_m. Decline in the zymogenous population in these experiments appears to occur after approximately two to three weeks incubation depending upon at least soil moisture conditions.

Organism numerical variation among similarly prepared and incubated microcosms was dependent upon taxonomic groups which in general may be ranked: fungi > nematodes > bacteria > actinomycetes, while the variation among the "integrator" variables, carbon dioxide production and oxygen consumption, was much less than the organism groups. It is anticipated that better control of soil/litter compaction, particle size, nutrients, and initial organism numbers and species would further greatly reduce variation among microcosms.

On the basis of this first experiment it appears that cadmium chloride amended soil markedly alters soil respiration. At low cadmium concentrations there was community stimulation of oxygen consumption as compared to carbon dioxide degeneration suggesting uncoupling of respiratory enzymes whereas high cadmium levels produced a general stoichiometrically equal reduction (40 percent) in both gas rates. The mechanism for the high level inhibition is unknown but information about excess heat

evolution from cadmium treated units would have been useful in confirming possible uncoupling.

The effect of cadmium treatment on organsism densities over the four-week duration of experiment indicated no numerical changes beyond those expected from inter-microcosm variations. It would be likely that upon further incubation, population densities shifts would be seen. Further, it would also be expected that successional events would be altered because of differential sensitivities of different organisms to the toxic effects of cadmium.

The reduction in respiratory activity brought on in cadmium amended soil would eventually be felt at the primary producer level when plant growth would be limited by the reduction of essential nutrient delivery rates as the result of inhibition of organisms performing organic remineralization in the soil.

It is concluded, that natural soil and litter effects,as well as "man-produced" effects such as cadmium amendment, may be successfully detected in soil microcosms using oxygen consumption and carbon dioxide generation. The use of heat generation remains problematical, but is a realistic goal.

SECTION II
INTRODUCTION

Decomposition is an essential process in all food webs if they are to remain in temporal equilibrium. The nature of the forest decomposition/primary production system is as a cyclic process in which primary produced organic matter in the form of litter fall, leachates, root exudates and sloughage is remineralized by soil macro-and micro-organisms (the decomposers) to plant nutrients in a series of "shredding" and solubilizing steps (McBrayer, Reichle, and Witkamp, 1974; Ausmus and Witkamp, 1974; Gist, 1972). The extent of this process is shown by the estimates that 80-90 percent of net primary production in terrestrial ecosystems is ultimately acted on by decomposer organisms (Odum, 1971; Witkamp, 1971; Wittaker, 1970), which along with the primary producers may make up to 95 percent of the total biomass in deciduous forests (Odum, 1971). It has been estimated that up to 70 percent of the caloric or biomass input to the decomposers is below ground from the roots of the primary producers (McBrayer, et al., 1974). It can be imagined that any interruption in the decomposition portion of this cycle might lead to a decrease in formation of readily utilizable essential plant nutrients that will subsequently limit plant growth rates.

Both natural and man modified environmental conditions in the soil and litter may markedly affect rates of decomposer activities. It is known that temperature, soil moisture, oxygen concentration, particle size, and quality and quantity of organic matter among others affect soil decomposition processes (in Gray and Parkinson, 1968). Relatively little is known about many man modified conditions, e.g., what are the effects of addition of the heavy metal cadmium as it accumulates as a contaminant of phosphate fertilization of agricultural lands (Williams and David, 1973). Further, many materials are known to stimulate organisms

at very low concentration and inhibit at higher concentrations (Loomis, 1971).

Cadmium is known to stimulate and/or inhibit plants, animals and microorganisms. In eucaryotic organisms, the action of cadmium is known to involve (1) blockage of the electron transfer from the TCA cycle to electron transport chain, (2) blockage of enzymes necessary for the synthesis of ATP by respiratory chain enzymes and (3) binding to the enzyme ATPase which is required to hydrolyze the reaction of ATP to ADP (Berry, Osgood, and St. John, 1974; White, Handler and Smith, 1973). It is thought that cadmium reacts with the sulfahydral groups in the affected enzymes. The mechanism of reaction appears to be the entrance of the cadmium ion (Cd^{2+}), the most active form of cadmium, into the cell where it binds to the membrane of the mitochondria. In certain yeasts, cadmium effects result in respiratory deficiencies related to the loss of "cristate" mitochondria producing petite colonies (Lindegren and Lindegren, 1973). In this case, cadmium might be thought of as a mutagenic agent. In procaryotic cells, both (deJong, 1971) neutral and inhibitory effects, depending upon cadmium concentration (Zwarum, 1973) were demonstrated on Azotobacter sp., Escherichia coli and other bacteria. For a review of pollutant stress effects on soil litter decomposers see Wiley (In preparation by U.S. E.P.A.).

In the study of effects of stressers on soil decomposition, particularly toxic substances such as cadmium and other airborne pollutants, it is difficult to "sort out" the treatment effect from the natural variation within and among soil horizons. Further difficulties often arise when it is necessary to clean up large land areas used for toxic substance studies upon completion of an experiment. The former problem was encountered by Bond, et al., (1974) who were unsuccessful in "sorting out" the effects of natural variation from the air pollutant treatments on different soil plots obtained from adjacent locations. To obviate the natural

variation confounding soil treatment experiments it was reasoned that if "identical" forest soil/litter simulating microcosms could be prepared and incubated that one might then be able to more readily detect the effects of stressor treatment on soil processes. This communication presents a description of the use of a coniferous forest soil/litter microcosm system, and how the microcosms responded to (a) similar preparation, (b) three levels of soil moisture, and (c) three levels of cadmium stressor.

SECTION III
METHODS

Three experiments with four soil/litter microcosms, one acting as a thermobarometer, were performed to obtain a preliminary evaluation of the soil microcosm technique under soil stressing conditions. The first experiment was performed to crudely observe variation between three "identical" microcosms, the second the effects of soil moisture on microcosm performance, and the third to test the effects of three levels of cadmium on the system performance. Microcosm performance was measured by so-called "integration" variables such as oxygen consumed, carbon dioxide produced and heat generated, and changes in "differentiated" variables such as microbial and invertebrate populations, and chemical changes in the soil/litter substrate.

Microcosm System Design and Preparation

Four soil microcosm systems similar to those used by McGarity, Gilmour and Bollen (1958) were constructed in four modular components: the soil/litter microcosm or reactor "insert" module, the reactor chamber module, the electrolytic oxygen generator module, and carbon dioxide trap module.

The soil/litter "insert" module was the microcosm proper and consisted of a plastic lined number 300 can layered with (starting from the bottom of the can), 0.5 cm glass wool, 150.00 gm of soil, 1/16 in mesh plastic screen, and 15.00 gm of litter sifted through 1/16th inch mesh screen. The soil and its overlying litter were obtained from a visually uniform area in a Douglas Fir forest stand in the Oregon Coast Mountain Range during the period June through November. Variation between up to 50 replicate microcosm "inserts", was minimized by sifting large amounts of homogenized (Waring Blended) and specifically prepared soil into all

similarly treated "inserts" simultaneously. Compaction of the soil was crudely achieved by tapping the bottom of the microcosm to a predetermined soil depth. The plastic screen was used to later facilitate soil/litter separation for destructive analysis.

The reactor chamber module consisted of a one quart Dewar flask large enough to accept the microcosm "insert." The flasks were closed off at the top with a number 17 neoprene stopper through which were inserted a small bore glass tube connected to the oxygen generator module, a thermoregulator (Scientific Supplies Company Catalog No. 61845-009), and wires to a heating resistor taped to the mercury bulb of the thermoregulator. The thermoregulator and heating resistor were electrically connected (Figure 1) so that a mercury switch would be thrown to turn on both an electric clock and the heating resistor when the temperature in the reactor was below the "set-point" of the thermoregulator. All of the reactors were maintained at 20°C in a constant temperature room set at 17 to 18°C. A heat loss and resistor heat constant must be measured and used to evaluate the heat production in each reactor. Biologically generated heat was calculated in calories using equation 1:

Biologically Generated heat from test microcosm (calories)	=	test/control reactor heat loss constant ratio	x	heat generated in control microcosm	-	Heat generated in test microcosm	(Eq. 1)

or

$$Q_{Test} = \frac{K_T}{K_C} X (R_C \times t_C) - (R_T \times t_T)$$

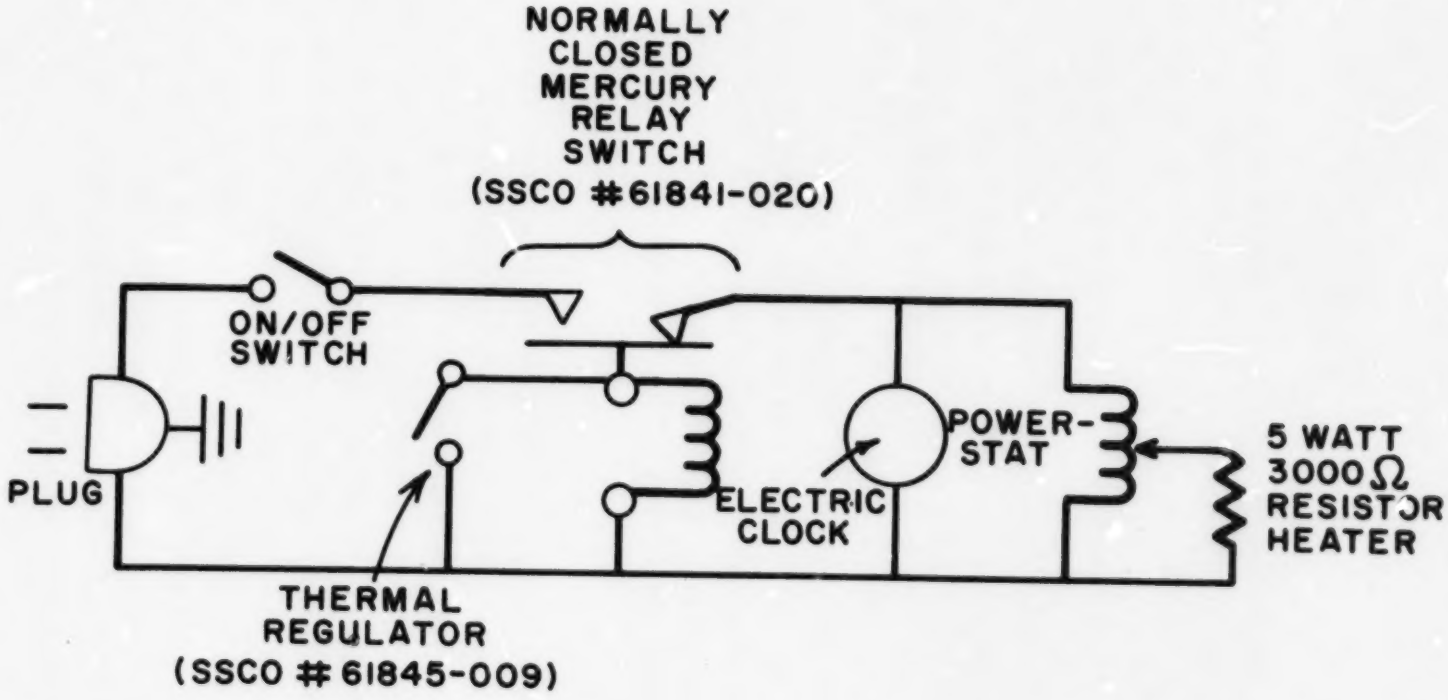

Figure 1 Wiring diagram for reactor module heat generating/regulation/monitoring system. SSCO stands for Scientific Supplies Company Catalog Stock number.

where

K_T = Heat loss rate constant (cal/hr) for the test reactor.

K_C = Heat loss rate constant (cal/hr) for the control reactor.

R_C = Heat production rate (cal/hr) constant for the control resistor.

R_T = Heat production rate (cal/hr) constant for the test resistor.

t_C = The time (hr.) the control resister was heating.

t_T = The time (hr.) the test resistor was heating.

Q_{Test} May be converted to calories produced per unit surface area and time by multiplication with appropriate factors.

Carbon dioxide within the reactor was trapped in 10.00 mls of 0.6 N NaOH held in a polyethelene vial hanging just above the "insert". Every 24 or 48 hours during an experiment the remaining alkali in the vial was titrated using the Coleman, et al., (1972) and Colman (1973) method and these data presented as milliliters carbon dioxide generated during the trapping period.

The oxygen consumed and carbon dioxide trapped during soil respiration in the closed reactor resulted in a reduced pressure that was transmitted to the electrolytic oxygen generator module (See Figure 2). Oxygen was generated when an electrolyte "switch" was closed at the positive electrode. Upon relief of the pressure by the generated oxygen the switch turned off. Hydrogen gas was generated at the negative electrode and trapped in an inverted and closed burette. One-half of the hydrogen gas volume is equal to the oxygen gas produced. A charcoal filter in the Tygon conducting tube between the oxygen generator and the reactor chamber absorbed toxic gases such as ozone and chlorine produced during the electrolytic process (Woodland, 1973). The electrolyte in the generator was an aqueous solution of 8 percent Na_2SO_4.

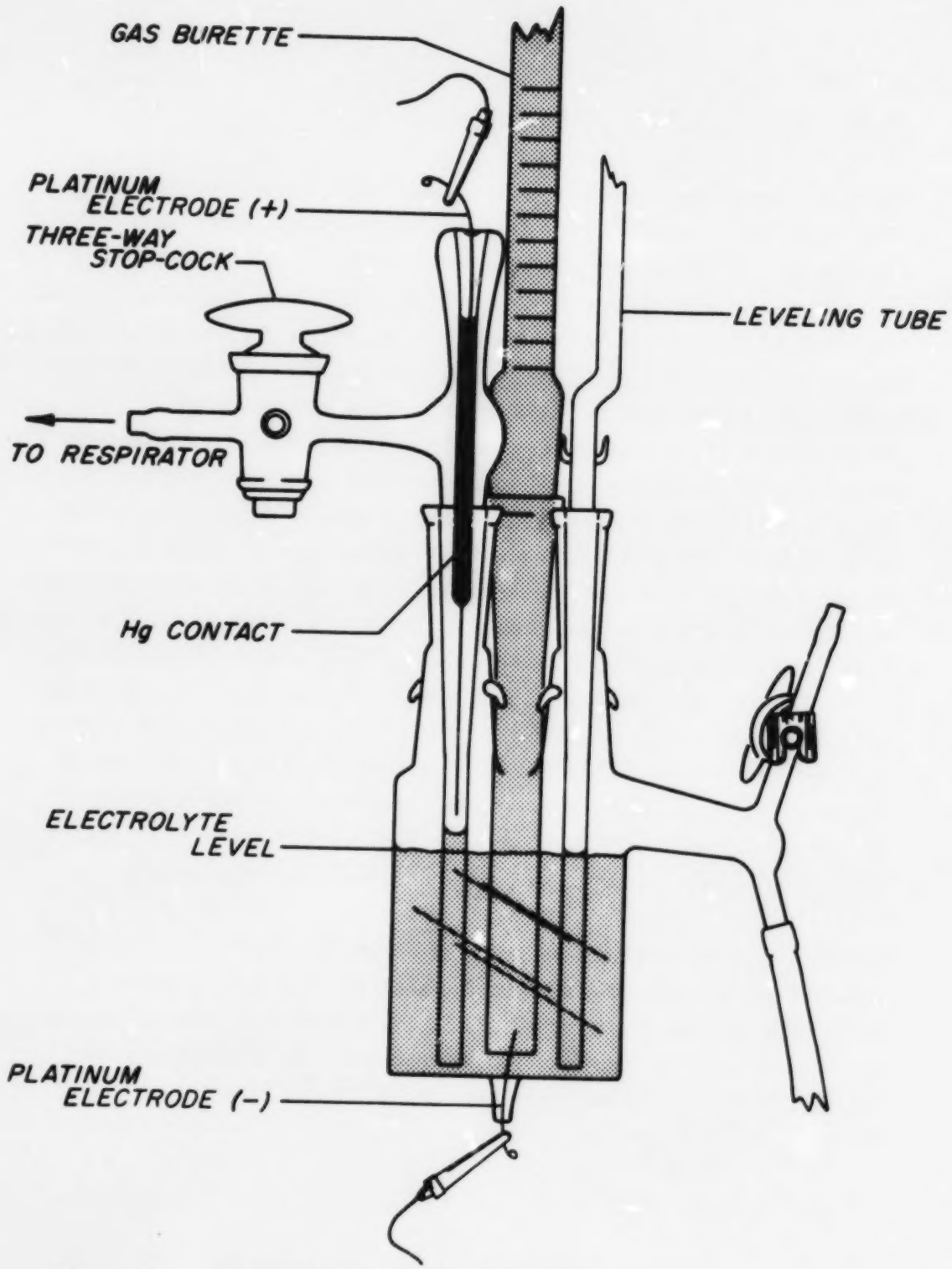

Figure 2. Diagram of electrolytic oxygen generator.

EXPERIMENTAL

The first experiment was designed to observe the variation between three "identically" prepared reactor inserts and incubation conditions. These inserts were prepared as above with the soil and litter adjusted to 21 and 44 percent soil moisture, respectively (Table 1). Incubation of the inserts in the reactors began immediately after their preparation, i.e., without any preincubation. The second experiment considered effects of soil moisture [measured as field water holding capacity (FWHC)]. Each of three sets of 16 inserts were simultaneously prepared. One set was prepared with 10 percent, the second set with 30 percent, and the third set with 60 percent of field water holding capacity of soil and litter. These inserts were preincubated for two weeks prior to random selection of one from each treatment and introduced into one of the reactors. The third experiment tested the effects of three levels of cadmium i.e., mean microcosm concentrations of 0.0, 0.01, and 10.0 milligrams cadmium as $CdCl_2$/kg of dry weight soil or litter. This experiment was commenced after two weeks preincubation at 20°C of "identically" prepared inserts. The $CdCl_2$ solution was injected into the soil and litter at 30 evenly spaced sites with a tuberculin syringe. The solution was made to a concentration that would just replace the 2-5 milliliters of evaporative water loss during the previous 2 days of the preincubation period.

Enough microcosm inserts were prepared at any one time to use in the reactors and for periodic destructive analysis during an experiment which were performed at circa 0, 3, 6, 12, and 24 days incubation after the inserts were introduced into the reactors. On the 24th day the reactor inserts themselves were also destructively analyzed.

SAMPLE ANALYSIS

Soil and litter moisture were determined gravimetrically on 105°C oven-dried samples. Surface spread plate counts of total heterotrophic bacteria, actinomycetes, and micro-fungi were counted on triplicate plates of 10-fold sterile phosphate buffer (APHA, 1970) dilutions of agitated soil and litter samples on the following three media: Acidified Potato Dextrose Agar (DIFCO 1953) for fungi, sodium albuminate agar (Pramer and Schmidt, 1964) for actinomycetes, and Bunt and Rovira's medium (1955) for bacteria. Incubation was at room temperature for two weeks.

Nematodes, rotifers and tardigrades, and other small metazoans were extracted from soil and litter samples with a modified Baermann funnel technique. Fifteen gm. soil and 1.5 gm. litter samples were wrapped in one-ply tissue paper and placed on a brass screen in a 13 cm diameter polypropylene funnel. The samples were flooded with tap water, and the nematodes were harvested after 6 days at 20°C. Nematode samples were counted immediately or stored at 6°C. Although the Baermann funnel is often inefficient, the small, thin samples used should result in effective extractions (Oostenbrinck 1971, Southey, 1970).

Microarthropods were recovered from 8-10 gm of litter and 100-120 gm of soil using a Tullgren technique with an apparatus similar to the high-gradient canister extraction of MacFadyen (1961). The "funnels" were heated with incandescent bulbs with the heat being progressively increased by use of a dimmer switch. For the present samples, the cover portion of the canisters were not air-conditioned as is usually the case for this type of apparatus. Specimens were collected into 70 percent ethanol (see also Bond, et al., 1974).

DATA ANALYSIS

Graphical and numerical methods were used to reduce the data. Reactor oxygen consumption, carbon dioxide and heat generation were summed for each reactor over time and the linear portions of computer drawn curves fitted by the least squares method to evaluate their rates of formation. Microbial data was plotted and a sample mean calculated for each analysis time.

SECTION IV
RESULTS

"IDENTICAL" MICROCOSM EXPERIMENT

In three "identically" prepared and incubated microcosms, the "integrator" indicators, rates of oxygen consumption and carbon dioxide production, decreased from a relatively more rapid near constant rate during the initial 200 hours after microcosm preparation to a slower near constant rate after 200 hours (Figure 3 and 4 and Table 1). Oxygen consumption for the three microcosms ranged from 0.239 to 0.215 ml $hours^{-1}$ with a coefficient of variation of 6.65 percent in the initial 200 hours and from 0.160 to 0.131 ml $hours^{-1}$ with a coefficient of variation of 9.93 percent in the final 350 hours. Also, during the first 200 hours incubation, carbon dioxide production ranged from 0.363 to 0.396 ml $hours^{-1}$ with a coefficient of variation of 5.03 percent while after this period the range was 0.190 to 0.176 ml $hour^{-1}$ with a coefficient of variation of 3.83 percent.

Bacteria, actinomycetes, and nematodes in the initial 150 hours of incubation increased in the litter 3, 30 and 2.5 times to 1.3×10^8, 2.5×10^7, and 7.5×10^1/gm (DW), respectively, and in the soil 4, 37 and 1.3 times to 6×10^1, 2.5×10^7 and 2.4×10^1/gm (DW) respectively (Figure 5), while the fungi decreased by 1/3 from 3×10^5/gm (DW) in the first 100 hours in the soil and litter, then returned to their initial value by 150 hours. The reason for this initial decrease and return in the fungi is unknown. For the remainder of the experiment, the bacteria and actinomycetes in the soil and litter, and fungi in the soil remained at the higher densities, while the nematodes in the soil and litter and fungi in the litter slowly decreased. Arthropods remained constant or increased slightly during the experiment.

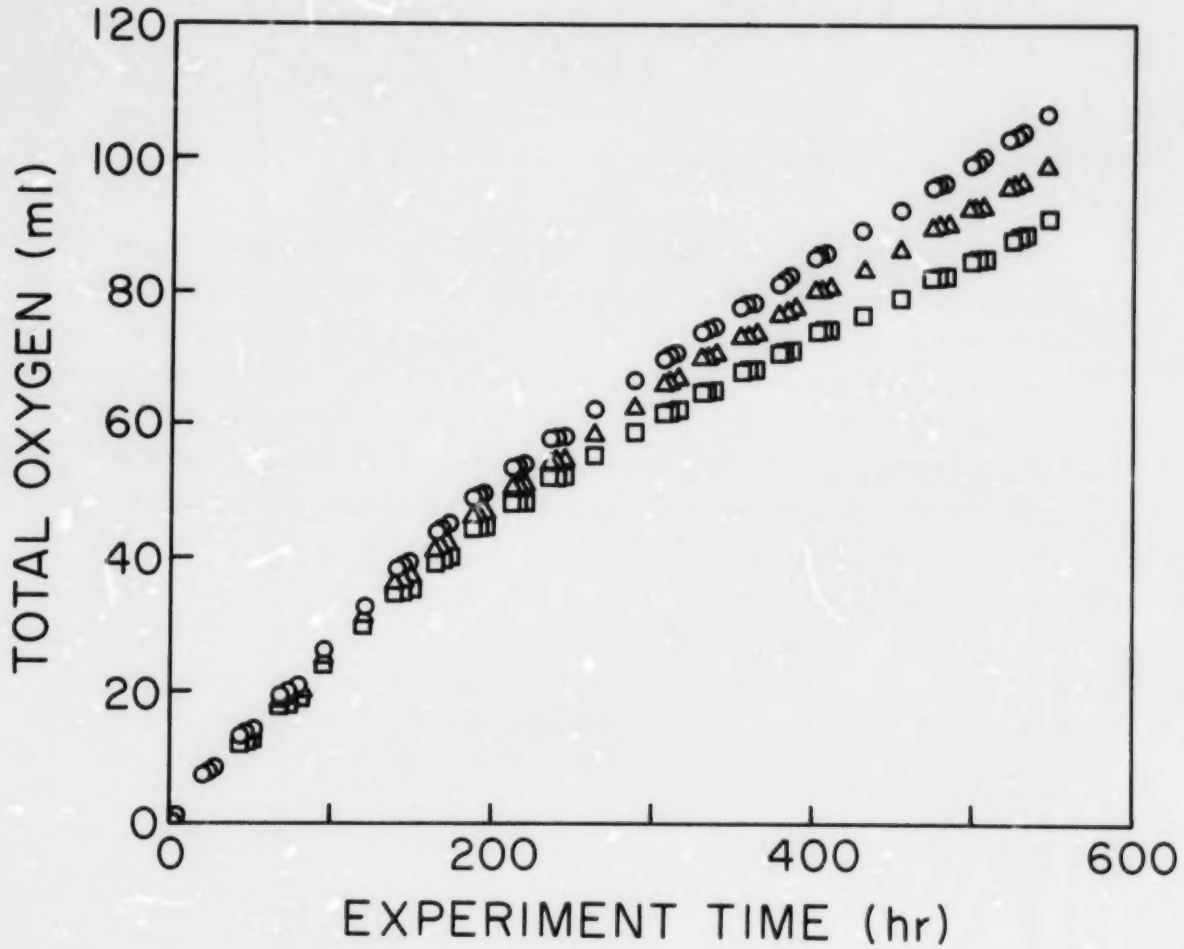

Figure 3. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms.

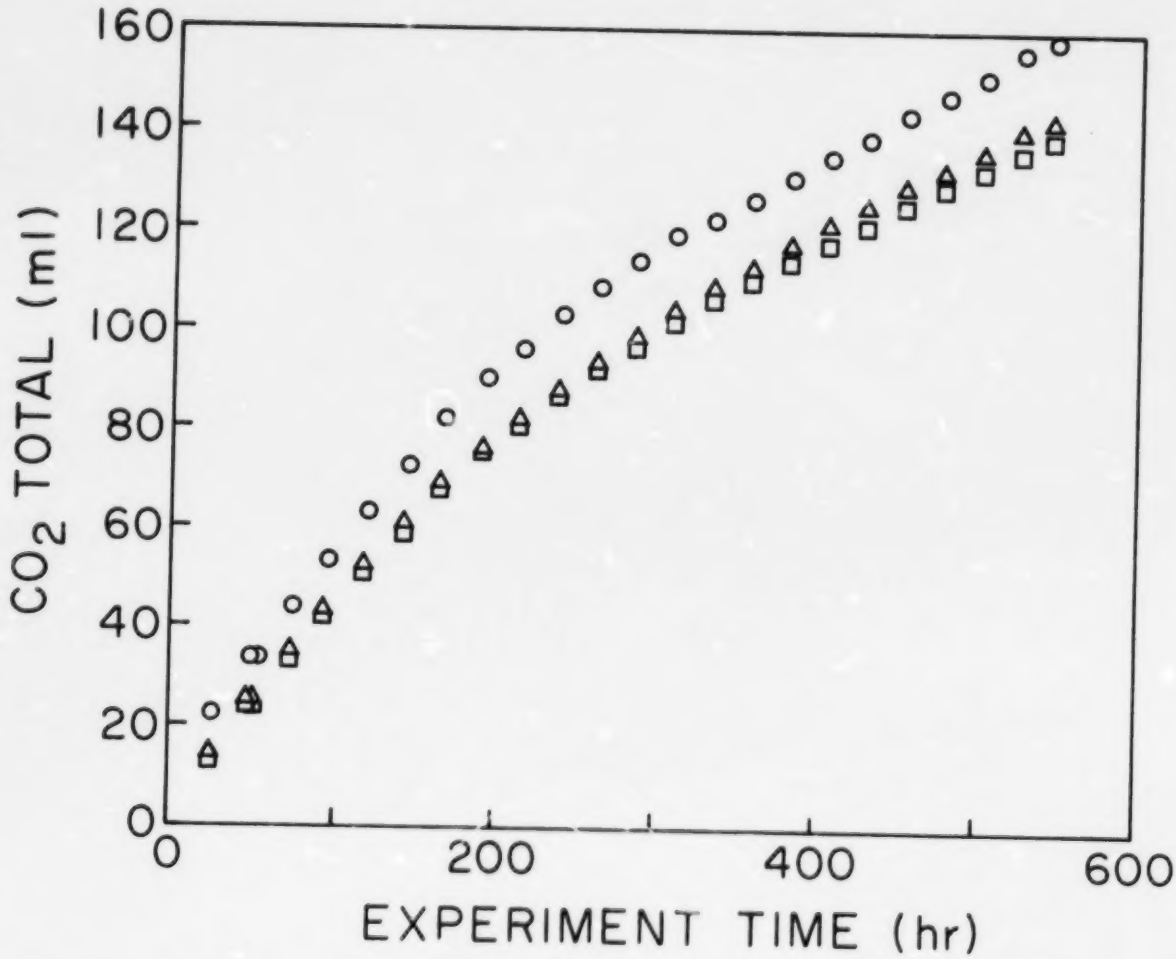

Figure 4. Accumulated carbon dioxide produced through time in three identically prepared and incubated coniferous forest soil/litter microcosms.

TABLE 1

TABULATION OF THE OXYGEN CONSUMPTION AND CARBON DIOXIDE GENERATION RATES FOR THE TWO PHASES (AGES) OF EACH OF THE 3 INDICATED EXPERIMENTS

Experiment Number	Treatment	Phase (Duration of Measurement in Days of Microcosm Age)	% Water (Soil/Litter)	Oxygen Uptake Rate (ml/hr)	Carbon Dioxide Generation Rate (ml/hr)	Coefficient of Variation (%)
1	None	0-14	21.0/44.5	0.249	0.396	CV_{CO_2} = 6.65
1	None	0-14	21.0/44.5	0.239	0.364	CV_{O_2} = 5.03
1	None	0-14	21.0/44.5	0.215	0.363	
1	None	14-24	20.5/37.2	0.160	0.190	CV_{CO_2} = 9.93
1	None	14-24	21.6/36.5	0.147	0.183	CV_{O_2} = 3.83
1	None	14-24	20.8/35.9	0.131	0.176	
2	Soil Moisture 10% of Field Capacity	14-26	15.6/20.8	0.028	0.054	N/A
2	Soil Moisture 30% of Field Capacity	14-26	20.5/47.2	0.076	0.123	N/A
2	Soil Moisture 60% of Field Capacity	14-26	26.9/58.3	0.123	0.195	N/A
2	Soil Moisture 10% of Field Capacity	26-38	14.7/43.5	0.039	0.104	N/A
2	Soil Moisture 30% of Field Capacity	26-38	19.3/34.9	0.046	0.077	N/A
2	Soil Moisture 60% of Field Capacity	26-38	26.2/49.5	0.133	0.147	N/A
3	None	14-20	16.8/38.0	0.140	0.251	N/A
3	Cadmium (0.01 ppm)*	14-20	16.8/38.0	0.178	0.256	N/A
3	Cadmium (10.0 ppm)*	14-20	16.8/38.0	0.131	0.210	N/A
3	None	20-38	18.8/35.4	0.137	0.157	N/A
3	Cadmium (0.01 ppm)*	20-38	18.6/33.0	0.140	0.137	N/A
3	Cadmium (10.0 ppm)*	20-38	17.4/32.6	0.081	0.101	N/A

*Theoretical final mean environmental concentrations.

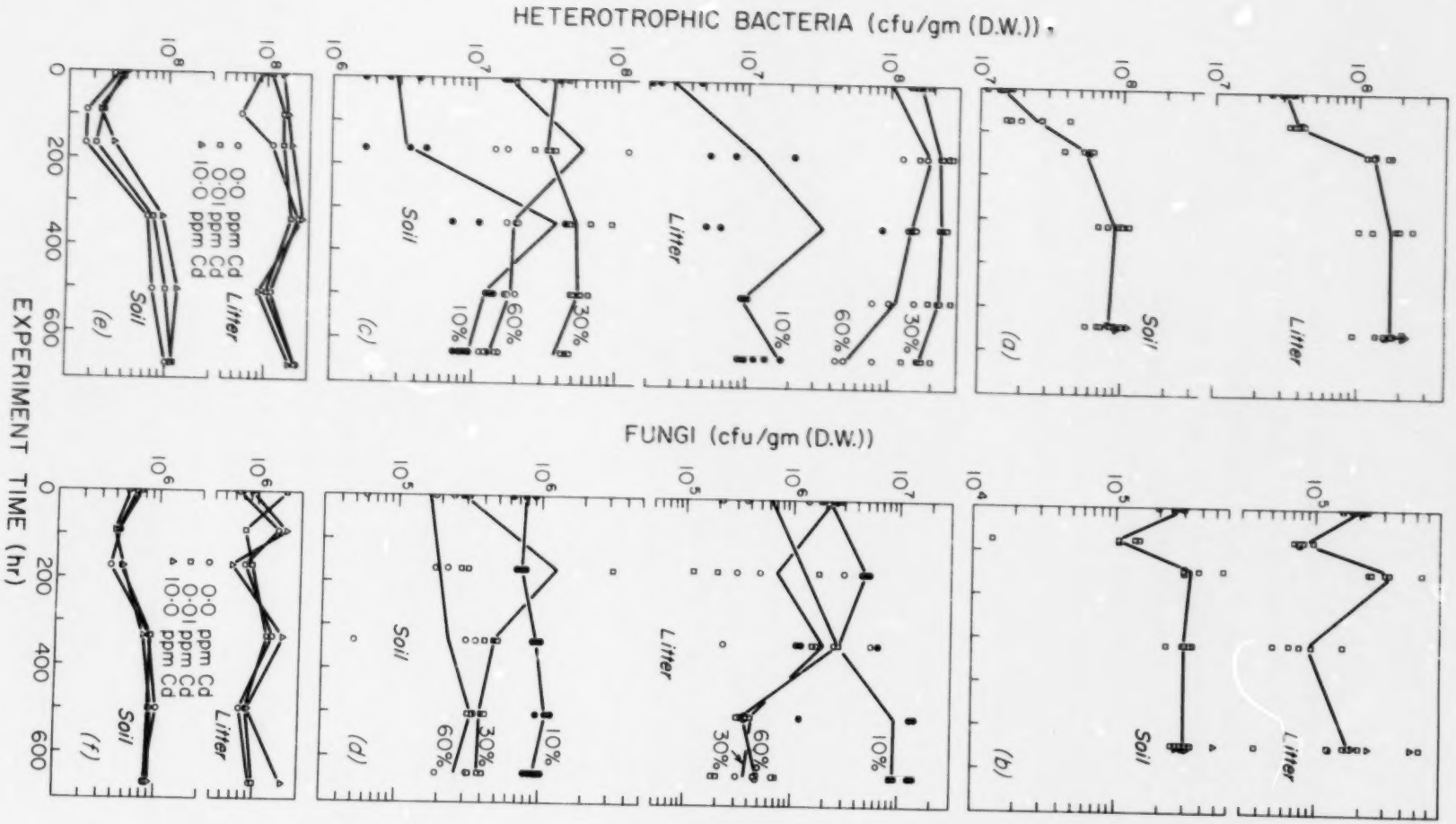

Figure 5 Graphs of heterotrophic bacterial and fungal propogules (CFU = colony forming units) in soil and litter in three "identically" processed microcosms (a & b); three "identically" processed microcosms incubated at 10, 30 and 60% of field water holding capacity respectively (c & d); and three "identically" processed microcosms injected with distilled water (0.0), 0.01 and 10.0 ppm final mean $CdCl_2$ concentration respectively (e & f).

The dispersion of values about the analysis times for counts from the indicated number of microcosms had a maximum coefficient of variation for either soil or litter at any given harvest time of 17.6 percent for bacteria (for 7 microcosms), 45.7 percent for actinomycetes (5), 52.4 for fungi (7), 39.2 percent for nematodes (9), and 75.2 percent for arthropods (5). In all cases for all three experiments in reactor incubated units terminal analysis measurements were scattered among the externally-incubated microcosms indicating close agreement between external and reactor incubated "insert" microcosms.

SOIL MOISTURE EXPERIMENT

The experiment concerned with soil moisture showed marked effects at both the "integrator" and organismal levels. As soil moisture increased, so did both oxygen consumption and carbon dioxide generation (Figures 6 and 7, Table 1). The rate of oxygen consumption over the duration of the experiment changed little for the 60 percent FWHC, but diminished by about 50 percent in the 30 percent FWHC microcosm and increased by 1/3 in the 10 percent FMHC microcosm. For CO_2 the 60 and 30 percent FWHC microcosms decreased 75 and 50 percent respectively and the 10 percent unit doubled its production.

The microbial population densities in replicate microcosms showed such a marked dispersion of counts that only general trends could be observed. (Figure 5). In both the litter and soil the numerically predominantly organisms were the bacteria and actinomycetes (in that order) in the 30 and 60 percent field holding capacity microcosms whereas the fungi were numerically dominant in the 10 percent units. The actinomycetes in both soil and litter in the 10 percent moisture microcosm and fungi in the 10 percent microcosm litter were the only microorganisms thought to be increasing in number over time.

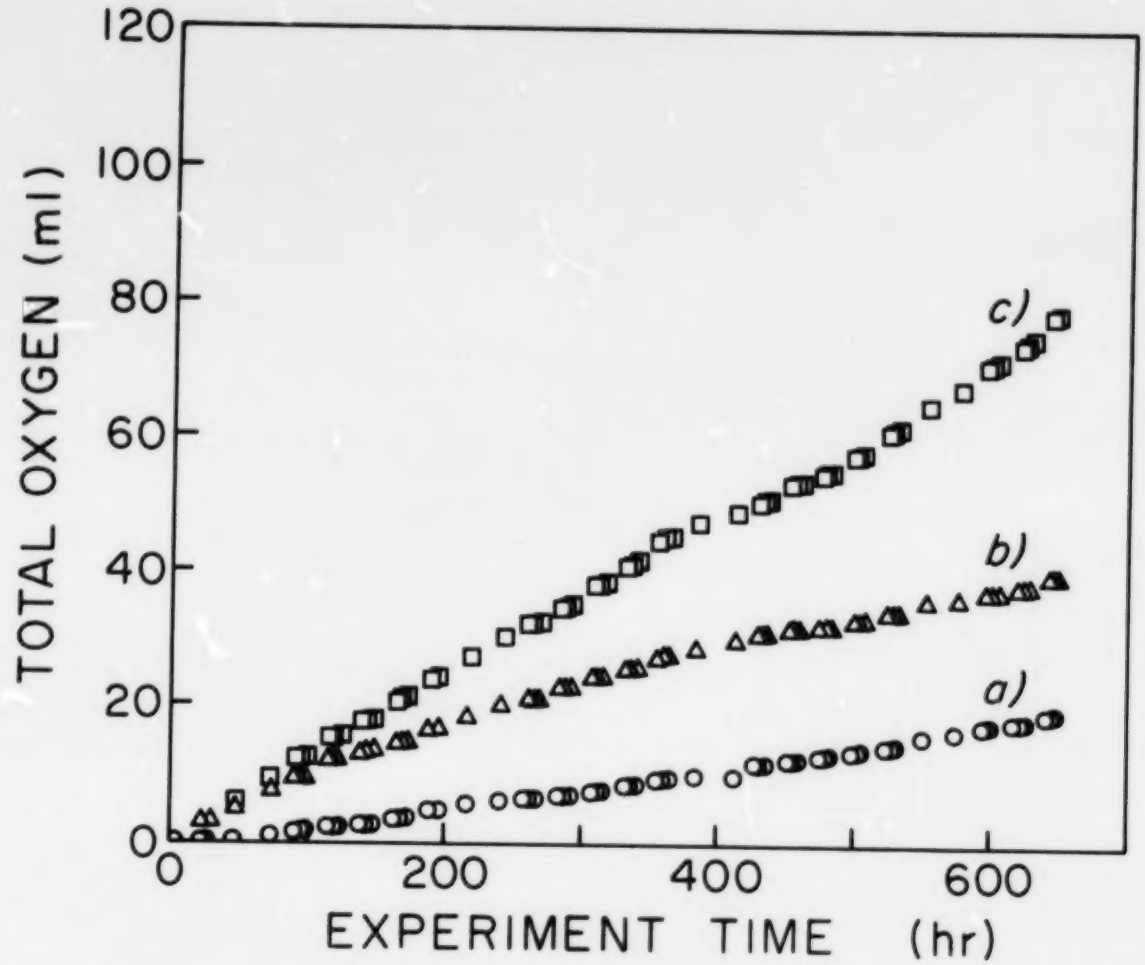

Figure 6. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to an initial soil moisture of (a) 10%, (b) 30%, and (c) 60% of field holding capacity.

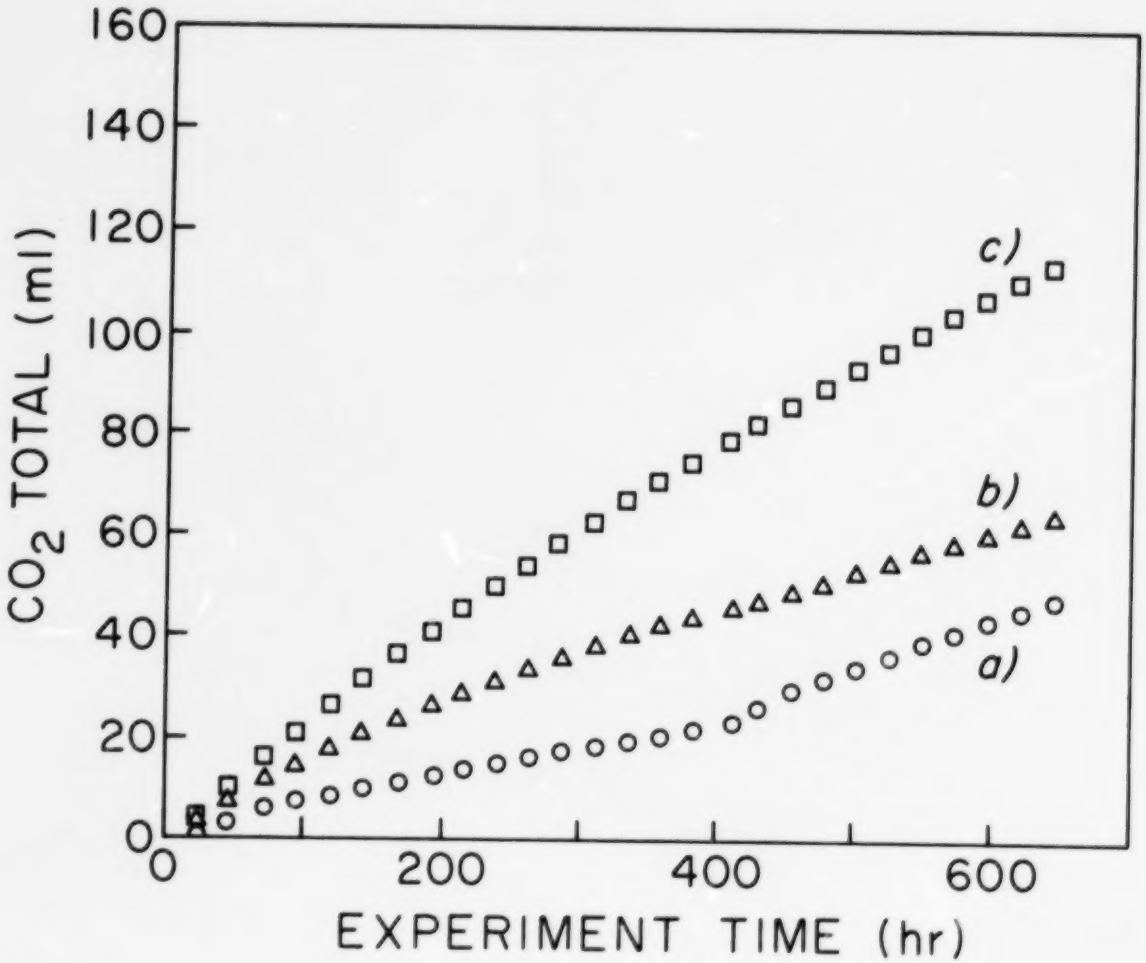

Figure 7. Accumulated carbon dioxide produced through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to an initial soil moisture of (a) 10%, (b) 30%, and (c) 60% of field holding capacity.

Nematode densities in the litter increased linearly with time at a rate of 0.0 nematodes/gm (DW)/day in the 10 percent FWHC microcosm, 0.17 nematodes/gm (DW)/day in the 30 percent and 10.8 nematodes/gm (DW)/day in the 60 percent FWHC microcosms. In the soil of these same microcosms, nematode densities increased from undetectable in the 10 percent unit to a plateau of 25 nematodes/gram (DW) after 300 hours incubation, after an initial 200-hour delay. Nematodes in the 60 percent soil moisture unit increased from 15 to 300 worms/gm (DW), and 30 percent showed no change from 40 worms/gm (DW).

Due to large variation between microcosms, arthropod populations were judged to showed no differences between treatments.

CADMIUM TREATMENT EXPERIMENT

Cadmium introduced into the microcosms produced detectable effects at the integrated oxygen consumption and carbon dioxide generation levels with no obvious effect at the organismal level (Figures 5, 8 and 9, Table 1). The 0.0 ppm cadmium control produced a constant oxygen uptake of between 0.140 and 0.137 ml O_2 hr^{-1}) during the initial and final 200 hours of the experiment respectively, while compared to the control, the 0.01 ppm cadmium microcosm showed a possible 27 percent stimulatory effect (0.178 ml O_2 hr^{-1}) during the initial 200-hour interval of the experiment, and the 10 ppm cadmium microcosm showed a 41 percent inhibitory effect (0.081 ml O_2 hr^{-1}) after the 200-hour time period.

Carbon dioxide production in all cases decreased throughout the duration of the experiment (Table 1). The 0.01 ppm cadmium microcosm treatment was very similar to the control rate, whereas the treatment 10 ppm

cadmium microcosm had not only a large O_2 consumption rate decrease but also a 36 percent reduction in CO_2 production compared to the control after the initial 200 hrs.

At the organismal level, frank cadmium treatment effects were not detectable in any group.

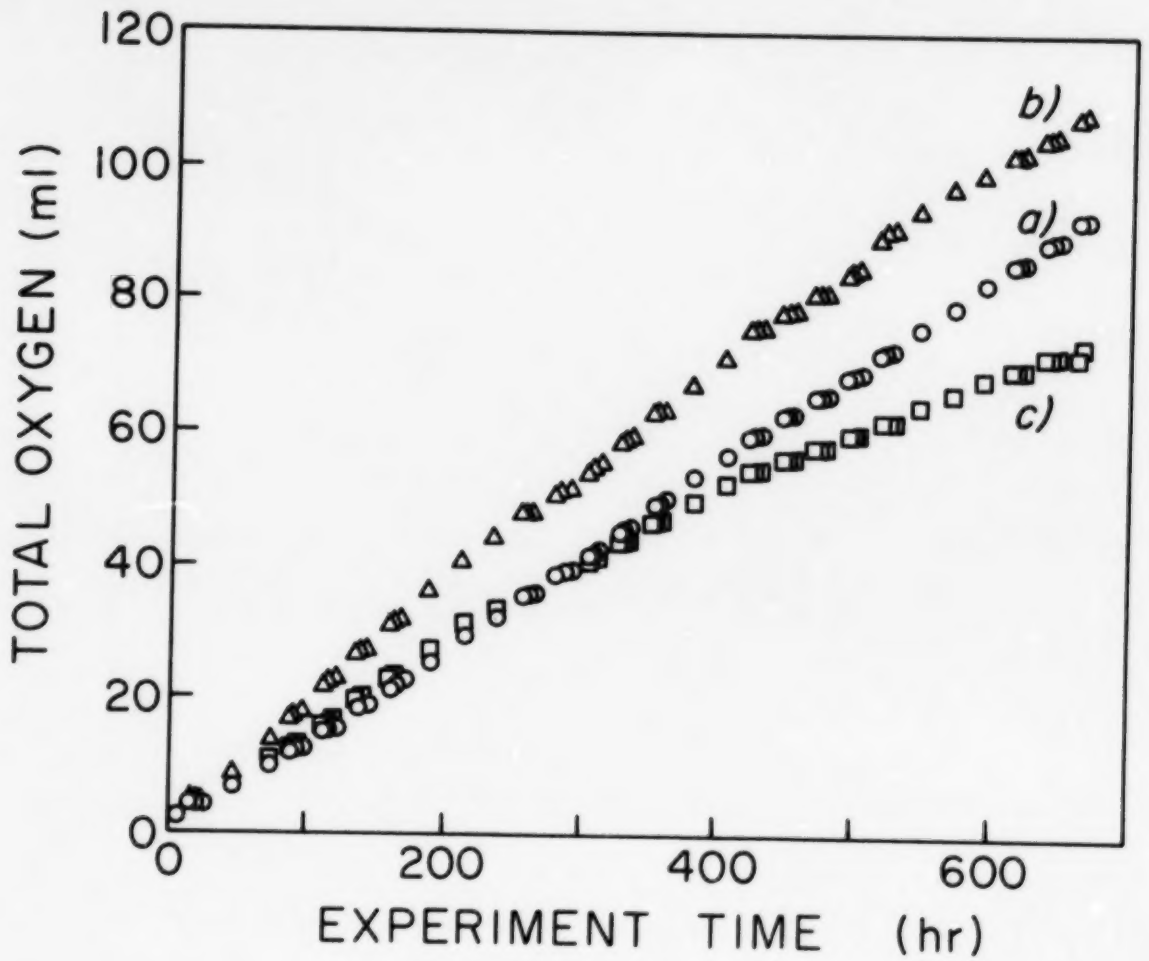

Figure 8. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to a final mean concentration of (a) 0.0, (b) 0.01, and (c) 10.0 ppm cadmium as chloride.

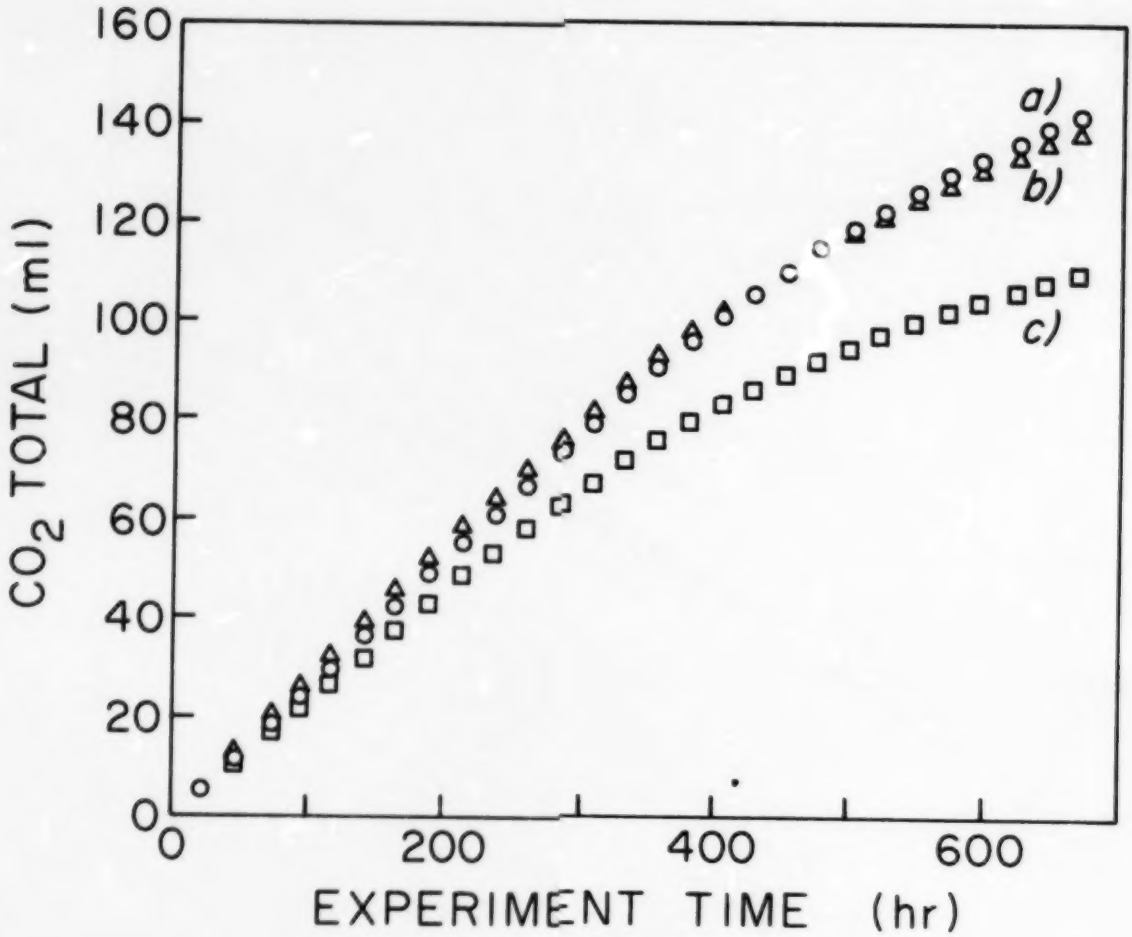

Figure 9. Accumulated carbon dioxide produced through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to a final mean concentration of (a) 0.0, (b) 0.01, and (c) 10.0 ppm cadmium as chloride.

SECTION V
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